## REMARKS/ARGUMENTS

Reexamination and reconsideration of this Application, withdrawal of the rejection, and formal notification of the allowability of all claims are earnestly solicited in light of the remarks that follow. Claims 1-55 are pending in the application.

All claims remain rejected under 35 U.S.C. 103(a) as being unpatentable over the combination of either U.S. Patent No. 6,231,881 to Usala or PCT Application No. WO 00/02999 in view of the Miller reference, and in further view of either the Davis or Pickart patents. Additionally, a majority of the claims remain rejected under the judicially created doctrine of obviousness-type double patenting as being patentable over the claims of U.S. Patent No. 6,261,587 in view of the Miller reference, and in further view of either Davis or Pickart. Applicant respectfully traverses these rejections.

The Examiner continues to argue that the Miller reference implies that "granulation and reepithelialization of an ulcer is desired," and further relies upon the Miller reference as teaching that agents that encourage wound healing can be used in the treatment of ulcers. The Examiner concludes that vascularization would be viewed, based on the Miller reference, as a beneficial treatment of ulcers because it is a "beneficial effect in granulation of tissue formation." The Examiner relies upon the Davis and Pickart references as teaching that blood vessel formation is an aspect of granulation tissue formation.

To establish a *prima facie* case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation either in the references themselves, or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest all the claim elements. The suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art and not based on the applicant's disclosure. *In re Vaeck*, 20 USPQ2d 1438 (Fed. Cir. 1991).

It is respectfully submitted that the Examiner has failed to establish a *prima facie* case of obviousness with respect to any of the rejections presented herein the Office Action. The Examiner cannot point to so much as a single sentence in the Miller reference that directly

suggests that an agent that encourages vascularization would be beneficial in an ulcer treatment regime. Instead, the Examiner has seized upon statements in Miller regarding the desirability of tissue granulation and boldly proclaimed, in essence, that the art suggest that any agent that encourages vascularization would be beneficial in ulcer treatment. In an attempt to further bolster this claim, the Examiner points to Davis and Pickart, which merely teach that blood vessel formation is one aspect of granulation tissue formation.

The art relied upon by the Examiner simply fails to credibly suggest that a vascularization-inducing agent would have a reasonable expectation of success as an ulcer treatment. There is no reference of record directly connecting a vascularization effect with successful treatment of an ulcer. There is also no reference of record that suggests that encouraging vascularization will lead to granulation of tissue. The Pickart and Davis references, as argued previously, only teach that blood vessel formation is one event that characterizes granulation of tissue. There is nothing to suggest in either reference that encouraging blood vessel formation will result in tissue granulation, meaning that the vascularization effect will necessarily also trigger the remaining wound healing processes, such as fibroblast activity or reepithelialization, that are necessary for tissue granulation. The art of record simply does not support the Examiner's statement that inducement of vascularization, by itself, would lead to tissue granulation or would be reasonably expected to be successful as an ulcer treatment.

If the Examiner's contention is correct, which Applicant does not admit, then one of ordinary skill in the art would view any vascularizing agent as a beneficial treatment for ulcers. However, the state of the art is clearly inconsistent with this conclusion. In fact, there is at least one known vascularizing agent that has been tried as a diabetic foot ulcer treatment and failed. In Chapter 18 of Levin and O'Neal's "The Diabetic Foot" (6th Edition), David Steed notes in Chapter 18 (entitled *Modulating Wound Healing in Diabetes*) that fibroblast growth factors (FGFs) "act as angiogenesis factors by stimulating growth of new blood vessels through proliferation of capillary endothelial cells" (p. 399). However, Steed goes on to admit that there are "no clinical trials that have proven FGF to be of benefit in clinical wound healing." A copy of the relevant portions of the above-cited reference is enclosed for the Examiner's review.

In addition, Applicants enclose a copy of the Richard *et al.* reference appearing in a 1995 issue of the journal *Diabetes Care*. In the Richard reference, a study of the use of FGF as a treatment for chronic neuropathic foot ulcers in diabetic patients is described. As noted on the first page (p. 64), the researchers noted that FGF performed no better than a placebo in reducing ulcer perimeter and area, and concluded that application of FGF has no advantage over a placebo for healing chronic neuropathic diabetic ulcers of the foot. Thus, Applicants have presented prior art evidence that is clearly contrary to the Examiner's conclusion that any vascularizing agent would be expected to succeed as an ulcer treatment. In fact, FGF is a vascularizing growth factor that has failed as a diabetic foot ulcer treatment. This clearly establishes that one of ordinary skill in the art, having benefit of the knowledge of the FGF failure, would not expect each and every agent capable of triggering vascularization to automatically find success as an ulcer treatment. In fact, one of ordinary skill in the art would be highly skeptical of such a claim. As a result, *prima facie* obviousness clearly cannot be established using the rationale and combination of references relied upon by the Examiner. In light of the foregoing, Applicants respectfully request reconsideration and withdrawal of all rejections based on a combination of the Usala references and the Miller reference.

**Consideration of Previously Submitted Information Disclosure Statement**

It is noted that an initialed copy of the Form PTO-1449 that was submitted with Applicants' Supplemental Information Disclosure Statement filed May 18, 2004 has not been returned to Applicants' representative with the Office Action. Accordingly, it is requested that an initialed copy of the Form PTO-1449 be forwarded to the undersigned with the next communication from the PTO. In order to facilitate review of the references by the Examiner, a copy of the Supplemental Information Disclosure Statement and the Form PTO-1449 are attached hereto.

It is not believed that extensions of time or fees for net addition of claims are required, beyond those that may otherwise be provided for in documents accompanying this paper. However, in the event that additional extensions of time are necessary to allow consideration of this paper, such extensions are hereby petitioned under 37 CFR § 1.136(a), and any fee required therefore (including fees for net addition of claims) is hereby authorized to be charged to Deposit Account No. 16-0605.

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I hereby certify that this paper or fee is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 on the date indicated above and is addressed to: Mail Stop AF, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450

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# Effect of Topical Basic Fibroblast Growth Factor on the Healing of Chronic Diabetic Neuropathic Ulcer of the Foot

## A pilot, randomized, double-blind, placebo-controlled study

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**OBJECTIVE** — To assess the efficacy and safety of topical human recombinant basic fibroblast growth factor (bFGF) on the healing of diabetic neurotrophic foot ulcers.

**RESEARCH DESIGN AND METHODS** — Seventeen diabetic patients suffering from chronic neuropathic ulcer of the plantar surface of the foot entered a pilot, randomized, double-blind study comparing local application of bFGF with placebo. Main inclusion criteria were a typical neuropathic ulcer of Wagner grade I–III, more than 0.5 cm in the largest diameter, with an abnormally high vibration perception threshold in the absence of significant peripheral vascular disease or wound infection. bFGF or placebo was applied daily during the 6 weeks as inpatients then twice a week for 12 weeks. Evolution of ulcer size was assessed through weekly clinical examination and computerized photographs.

**RESULTS** — In the bFGF group, three of nine ulcers healed compared with five of eight in the placebo group (NS). The weekly reduction in ulcer perimeter and area was identical in both groups, as was the rate of linear advance from entry to the 6th week of treatment (bFGF: 0.053 ± 0.048 mm vs. placebo: 0.116 ± 1.129 mm): the same result was obtained at the 11th week. Moreover, percent healed area at the end of the study did not differ significantly. No side effects were observed during bFGF application.

**CONCLUSIONS** — Topical application of bFGF has no advantage over placebo for healing chronic neuropathic diabetic ulcer of the foot. Because diabetes causes significant wound-healing defects, we hypothesized that using a single growth factor might be insufficient to accelerate wound closure of diabetic ulcers.

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bFGF, basic fibroblast growth factor; ABI, ankle-brachial pressure index; PDWHF, platelet-derived wound healing formula; PDGF, platelet-derived growth factor.

Foot lesions are a major problem in people with diabetes mellitus, in terms of morbidity, mortality, disability, and financial cost (1). In diabetic patients, nonhealing foot ulcers, mainly due to peripheral and autonomic neuropathy (2–5), frequently lead to lower limb amputations (6,7). Numerous approaches using topical treatment have been proposed, including antibacterial agents (antibiotics, antiseptics), various types of dressings, powders, pastes, and creams (2,3). But most ulcers respond poorly to this conventional treatment, often requiring a long hospital stay (8), and the recurrence rate is high (9).

Wound healing is a complex process involving highly regulated responses of specialized cell types, in which locally secreted growth factors seem to play a key role (10). Recently, the availability of large quantities of growth factors has allowed researchers to test their therapeutic use as topical agents to accelerate wound healing (11).

Considering the impairment of wound healing in diabetes mellitus and the role of basic fibroblast growth factor (bFGF) in the healing process clearly suggested by experimental in vivo study (12,13), we conducted for the first time a pilot (phase I and II) randomized, double-blind, and placebo-controlled study to evaluate the efficacy and safety of topically applied recombinant bFGF in stimulating the repair of diabetic neuropathic foot ulcers.

## RESEARCH DESIGN AND METHODS

— After obtaining informed consent, 17 diabetic patients aged 39 to 73 years, were entered into the study. They were recruited from two departments of diabetology at Montpellier University Hospital ($n$ = 7) and at Le Grau du Roi Medical Center ($n$ = 10). All but one had non-insulin-dependent diabetes mellitus. The experimental protocol in accordance with the Declaration of Helsinki was approved by the French National Ethical Committee.

Patients were included if they had a typical, chronic, nonhealing, neuropathic ulcer on the plantar surface of the foot, grade I-III, according to Wagner's classification (14); this classification system was chosen because it appears valuable in evaluating the outcome of diabetic foot ulcers (15). After mechanical excision, the largest part of the wound had to measure more than 0.5 cm. The neuropathic nature of the ulcers was confirmed by biothesiometry (16) with a vibration perception threshold higher than 30 V either at the great toe or at the medial malleolus (Biomedical, Newbury, OH). Moreover, no patient had significant peripheral vascular disease, as evidenced by Doppler wave form analysis; ankle-brachial pressure index (ABI) was not used, as most neuropathic patients had falsely elevated ABI values due to medial arterial calcification (17). Before patients entered the study, active infection was ruled out by clinical examination and X rays, and a tight glycemic control was obtained by intensive insulin therapy using three subcutaneous injections a day or continuous subcutaneous insulin infusion (Mark II Infusers, Novo Nordisk, Boulogne-Billancourt, France); intensive insulin therapy was maintained during the entire experimental period and patients were instructed to check their capillary blood glucose level three to six times a day.

After randomization, patients were treated with either placebo (normal saline) or bFGF (Farmitalia Carlo Erba, Milano, Italy) until the ulcer was completely healed or the patient completed the 18 weeks of the treatment. The treatment period was divided in two consecutive sequences: during the first 6 weeks, treatment was applied once a day on an inpatient basis, whereas during the last 12 weeks, it was applied twice a week, and patients were allowed to return home if the ulcer progression was satisfactory. Patients were encouraged to keep the foot with the ulcer totally nonweight-bearing until the study was completed, but no specially designed footwear was prescribed.

**Table 1—*Baseline characteristics of the patients***

| | Group P (Placebo-Treated) n = 8 | Group F (bFGF-Treated) n = 9 |
|---|---|---|
| Age (years) | 63.6 ± 7.9 | 61.9 ± 10.0 |
| Sex (M/F) | 7/1 | 9/0 |
| Body mass index (kg/m$^2$) | 29.3 ± 2.6 | 26.4 ± 4.6 |
| $HbA_{1c}$ (%) | 7.1 ± 1.7 | 7.9 ± 1.7 |
| Fructosamine (mmol/l) | 284.4 ± 42.2 | 295.1 ± 75.0 |
| Duration of diabetes (years) | 18.8 ± 9.5 | 20.9 ± 12.3 |
| Vibration perception threshold (V) | 37.3 ± 14.9 | 46.3 ± 6.4 |
| Ulcer duration (months) | 27.9 ± 42.2 | 22.4 ± 27.9 |
| Ulcer largest diameter (mm) | 18.1 ± 6.2 | 18.0 ± 12.0 |
| Wagner's classification | | |
| Grade 1 | 1 | 2 |
| Grade 2 | 4 | 4 |
| Grade 3 | 3 | 3 |

Data are means ± SD. No statistically significant difference was found between groups P and F. Vibration perception threshold was measured at the great toe.

After randomization, eight patients were treated with placebo (group P) and nine received bFGF (group F). There were no significant differences in patient characteristics at entry into the study (Table 1). Glycemic control was similarly good in the two groups, due to the initiation of the intensive insulin therapy before inclusion in the protocol. The distribution in Wagner's grades and the initial size of ulcers did not differ between groups P and F.

bFGF was produced by recombinant DNA technology using *Escherichia coli* type β. A stable pharmaceutical formula was obtained, having a potent biological activity both in vitro and in vivo. From a lyophilate containing 50 μg of bFGF, formula was reconstituted with saline at a final concentration of 5 μg/ml in a vial connected in a sterile manner to a special spray delivery system: a volume of 50 μl containing 500 ng of compound was delivered at each pressure, so that at 2 cm from the skin, the device delivered spray on a 4.15 cm$^2$ area. When reconstituted, the solution was kept at 4°C for no more than 3 days. After mechanical removal of necrotic tissue and excision of surrounding callosities, placebo or bFGF was sprayed on the ulcer. According to the ulcer size, one or two sprays were delivered. Afterward, the ulcer was covered with a sterile petrolatum-impregnated gauze (Tulle gras Lumière, Sarback, Suresnes, France) in Le Grau du Roi center or dry compresses at Montpellier, until the next foot care. No antiseptics or special dressings were used.

Patients were evaluated each week: the length and width of the ulcer were measured and a photograph was taken, using an instant camera with a magnification of 1:1 (ImagePro, Polaroid, Cambridge, MA). The ulcer and perimeter area were evaluated for infection and treatment tolerance. At the end of the study, each photograph was coded and digitized (Mac IIx computer, Epson GT 4000 scan, and Epscan Mac 102 software); using Canevas software, ulcer perimeter and area were measured by one of us (J.P.D.), unaware of the patients' identity, date of photographs, and nature of the treatment. Measurements were standardized in reference to the distance between the base of the big and the fifth toe. The rate of wound healing was calculated from the change in wound perimeter and area; the rate of linear advance of healing

toward the ulcer center (δ, in mm) was calculated according to Gilman's formula (18).

Clinically, improvement of ulcer aspect was defined by a decrease of Wagner's grade, worsening by an increase and healing by grade 0.

At entry and at the end of the study, blood was withdrawn for determination of glycosylated hemoglobin, fructosamine, and hematological and biochemical routine parameters.

Statistical analysis was performed using BMDP statistical software (19). Fisher's exact test and the nonparametric Kruskal-Wallis test were used to compare qualitative and quantitative data, respectively, between groups. In each group, comparisons of data at entry and at the end of the study were carried out using Wilcoxon's exact rank-sum test and McNemar's test for dependent quantitative and qualitative data, respectively. Multivariate analysis of variance for repeated measures was performed to determine the effect of treatment and placebo on changes in standardized wound area over time. Times until 25 and 50% healing were analyzed according to Kaplan-Meier estimates of the healing time distribution; Breslow-Mantel's test was used to adjust for the subset of the prognosis variables. The stepwise Cox proportional hazards model was used to identify and control for these prognosis variables, when statistically significant. Linear correlation and analysis of covariance were performed to control for the effect of the initial wound diameter on Gilman's parameter.

**RESULTS** — During the study, good glycemic control was maintained in both groups; at the end of the study, the level of glycosylated hemoglobin was 6.4 ± 1.1% in group P and 7.5 ± 1.8% in group F (NS) and that of fructosamine was 281 ± 48 mmol/l in group P vs. 311 ± 40 mmol/l in group F (NS). These levels were not significantly different from those at entry.

In placebo-treated patients, five of eight ulcers (63%) healed completely compared with three of nine (33%) in the bFGF-treated group (NS) (Table 2). In seven of eight healed ulcers, healing occurred before the end of the study. Infection occurred on two ulcers in both groups, requiring the study to be stopped prematurely. One bFGF-treated patient refused to continue the study despite improvement in her ulcer status. In five patients, the study was conducted to the end: two ulcers improved but only one in group F healed completely. No significant difference was found between the two groups with regard to the duration of treatment. In addition, the number of patients having pursued the study beyond the 6th week was identical in groups P and F. The clinical evolution of the ulcers was not significantly different; repartition in Wagner's classification was similar in both groups at the end of the study.

According to standardized measures from photographs, no significant difference was found between the two groups for ulcer sizes at any week. In both groups, ulcer perimeter and area decreased significantly during the study. Decrease of ulcer perimeter and area was greater in group P, as was the daily reduction, but the difference was not statistically significant (Table 3). The same trend was also found when reduction of ulcer size was expressed as percentage of initial size (Tables 2 and 3).

Kaplan-Meier curves of times needed for ulcers to achieve 50 and 100% healing were plotted. Cox regression indicated no statistically significant effect because of treatment groups, centers, body mass index, patients' age, or ulcer duration. Mean healing times were identical for both groups (Table 2), and the median for 50% healing was 9.3 ± 2.1 and 5.8 ± 0.4 weeks for groups F and P, respectively (NS). Median time to 100% healing could not be compared because of the few events (<50% of ulcers com-

**Table 2—*Ulcer evolution during treatment***

| | Group P ($n$ = 8) | Group F ($n$ = 9) |
|---|---|---|
| Treatment duration | 64.8 ± 29.5 | 87.7 ± 38.0 |
| Clinical outcome | | |
| Healing | 5 | 3 |
| Improvement | 1 | 2 |
| No progression | 0 | 2 |
| Worsening | 2 | 2 |
| Ulcer perimeter reduction (% of initial perimeter) | 47.2 ± 36.4 | 35.8 ± 49.6 |
| Time for 50% of healing (weeks) | 5.8 ± 0.4 | 9.3 ± 2.1 |

Data are means ± SD. No statistically significant difference was found between groups P and F.

**Table 3—*Ulcer area reduction in the study groups***

| | At entry ($cm^2$) | At the end ($cm^2$) | Reduction ($cm^2$) | Reduction (% initial area) | Daily reduction ($mm^2$/day) |
|---|---|---|---|---|---|
| Group P | | | | | |
| Mean | 0.54 | 0.27 | 0.31 | 75.0 | 0.56 |
| SD | 0.37 | 0.54 | 0.24 | 39.1 | 0.52 |
| Group F | | | | | |
| Mean | 0.69 | 0.49 | 0.23 | 59.3 | 0.38 |
| SD | 1.27 | 1.14 | 0.20 | 44.5 | 0.54 |

No statistically significant difference was found between groups P and F. In cases of enlargement in wound size reduction was considered to equal 0.

pletely healed) at the end of the study in group F.

The rate of linear advance of healing was positive in the two groups, averaging 0.116 ± 0.129 and 0.053 ± 0.048 cm at 6 weeks (NS) and 0.184 ± 0.190 and 0.072 ± 0.089 cm at 11 weeks, for groups P and F, respectively ($P = 0.08$ by Kruskall-Wallis test). No significant relationship was found between initial ulcer size and rate of linear advance of the ulcer margins.

Topical bFGF treatment was well tolerated; we observed no clinical drug-related adverse events or abnormalities in hematological or biochemical data.

**CONCLUSIONS**— The main conclusion of this study is that local application of topical bFGF is no more effective than placebo to promote healing of neurotrophic diabetic ulcer of the foot, whether healing was expressed functionally (by Wagner's grade) or quantitatively (by measurement from photographs); moreover, the only trends we found showed a beneficial effect of placebo. The small size of the groups might have obscured a positive effect of bFGF, but an acceleration of wound healing in diabetic patients was recently reported using topical application of other growth factors in a sample of comparable size to this study (20). Of the eight healed ulcers (five in group P, three in group F), seven occurred during the hospital stay, when all patients were at bed rest, emphasizing the importance of keeping weight off of ulcer sites in promoting healing of neuropathic ulcers (2–4); this simple measure probably accounts for the good results obtained with placebo alone. A difference in compliance to non-weight-bearing recommendations between the two groups after hospital discharge seems unlikely to account for the failure of bFGF to accelerate healing. Moreover, the number of patients studied beyond the 6th week as outpatients was identical in the two groups, and the only ulcer that healed during the outpatient period was observed in group F. Diabetes control also is essential to promoting ulcer healing (2). In this study, blood glucose control was similarly good in both groups as evidenced by glycohemoglobin and fructosamine levels; thus, the deleterious role of external factors, mechanic or metabolic, can be ruled out in accounting for the poor results with bFGF.

The result of this trial is rather disappointing, considering the physiological role of bFGF in the healing process and the previous in vivo and in vitro experiments using bFGF.

Diabetes mellitus is characterized by failure of wounds to heal. It has been hypothesized that this defect may result from the decrease or unavailability of growth factors at the ulcer site (21). Thus, topical application of growth factors seems to be a logical means to promote wound repair in diabetic patients. Among growth factors, one of the most widely studied is bFGF, a heparin-binding single-chain peptide of 146 amino acids, with a ubiquitous distribution in mesoderm- and neurectoderm-derived tissues (13). bFGF is a potent mitogen for all cell types involved in the wound-healing process and is highly angiogenic and also chemotactic for fibroblasts and endothelial cells (22). Thus, bFGF appears to play a major role in wound healing, acting at almost all the steps of this complex process, and may represent a healing-promoting agent of potential value.

In vivo studies in uncompromised animal models showed that topical bFGF did accelerate healing (12,23). More relevant to our study are the results obtained in diabetic animals. In *db/db* mice, it was reported that topical application of bFGF resulted in an improvement in tissue repair (24,25).

Clinical trials on the effect of growth factor application on the healing of chronic diabetic wounds remain scarce and controversial; the most extensive clinical experience dealt with the so-called platelet-derived wound-healing formula (PDWHF), a homologous blood platelet releasate containing several growth factors, mainly platelet factor 4, platelet-derived growth factor (PDGF), transforming growth factor β, and β-thromboglobulin–related peptides (26). In randomized, double-blind, placebo-controlled trials, topically applied PDWHF significantly accelerated the wound-healing rate of chronic diabetic, mainly neurotrophic, foot ulcers (20,26). In contrast, topical PDGF applied in 25 diabetic patients with neuropathic ulcers did not result in a significant acceleration of the healing rate (27). In addition, it was recently reported that bFGF enhanced slightly, but nonsignificantly, healing of venous and diabetic ulcers in a double-blind, randomized trial (31). Thus clinical trials using growth factors have shown discrepant results. The poor effect of bFGF in the present trial may be explained in the light of the conflicting data of these previous studies: the topical application of a single growth hormone would be insufficient to promote healing in diabetic neuropathic ulcers, as suggested by some experimental data (29). Moreover, it was reported that topically applied epidermal growth factor and insulin acted synergistically to improve wound healing in diabetic rats (30). Thus, if a single growth factor may accelerate tissue repair in the absence of profound healing defect (32,33), a "cocktail" of several growth factors might be required to induce the same beneficial effect in chronic healing deficiency states such as diabetes mellitus.

The dose of bFGF locally delivered on ulcer sites may also be questionable. In this study, doses ranging from 0.25 to 0.75 $\mu g/cm^2$ were delivered at each application on the ulcer site, lower than those used in the successful treatment of pressure sores (29). Nevertheless, the dosage used in our trial was based on previous animal studies demonstrating the efficacy of such doses to promote healing in various clinical conditions, including corneal ulcers (33). On the other hand, bFGF was applied only twice a week during the last 12 weeks, which may explain the poor result beyond this time point. From this point of view, it is

worth noting that the efficacy of PDWHF in healing diabetic foot ulcers was obtained by daily application of the formula for 20 weeks (20,26). The last point deserving consideration is the galenic formulation of bFGF, given the fact that the product we applied was a simple collyrium. As bFGF, it might be locally degraded and/or adsorbed into the dressing, losing part of its eventual efficacy. Indeed, it was recently reported that the concentration of bFGF, in the same formulation we used, decreased by ~50% 4 h after contact with sterile gauze (34). Thus, it is possible that incorporating bFGF into a gel or a cream might result in a significant effect.

In summary, the results from this pilot study do not demonstrate significantly improved healing of neuropathic ulceration of the foot in diabetic patients treated by topically applied bFGF compared with placebo. The lack of a beneficial effect of bFGF might be explained by the profound healing defect in diabetes mellitus, requiring the combination of several locally applied and specially formulated growth factors on a daily basis.

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# THE DIABETIC FOOT

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Chapter 18

# MODULATING WOUND HEALING IN DIABETES

■ David L. Steed

Diabetic foot ulcers are a serious problem that may lead to infection and loss of limb. There are between 11 and 16 million patients with diabetes in the United States. Ten percent of diabetic patients may be at risk for the development of foot ulcers. Although 6 to 10% of hospitalizations in diabetic patients are for management of foot ulcers, these admissions account for nearly 25% of the hospital days in this patient group. There are 50,000 to 60,000 amputations performed in diabetic patients each year in the United States. The cost to society of caring for these patients is billions of dollars.

Diabetic foot ulcers occur because of neuropathy and peripheral vascular disease. Sixty to 70% of diabetic patients with foot ulcers have peripheral neuropathy as the etiology, while 15 to 20% have peripheral vascular disease as the cause, and another 15 to 20% have both. The neuropathy occurs as a complication of prolonged glucose elevation. It may be present in as many as 10% of patients when diabetes is diagnosed and nearly half of diabetic patients who have had their disease for more than 20 years. Peripheral neuropathy has both sensory and motor components. The sensory neuropathy leads to a loss of protective sensation in the foot. The motor neuropathy affects the nerves that control motion of the foot. Paralysis of the intrinsic muscles of the foot leads to a "claw" deformity of the foot where the toes are pulled up and do not touch the ground. This causes the metatarsal heads to become more prominent on the plantar surface and reduces weight bearing from the toes. The metatarsal heads are a common site of plantar ulceration in diabetic patients. This is especially true for the areas beneath the first and fifth metatarsal heads. The patients can also develop midfoot collapse with loss of the plantar arch due to fractures and fracture dislocations. This Charcot neuroarthropathy may result in a markedly abnormal shape to the foot, which coupled with the sensory neuropathy, makes the diabetic patient at risk for skin breakdown.

There are other factors that lead to ulceration in diabetic patients. The patients commonly have peripheral vascular disease. Diabetes is a commonly accepted risk factor for atherosclerosis as are smoking, hyperlipidemia, and hypertension. Although there was once debate as to whether diabetic patients had "small vessel disease" with occlusion of very small vessels, that theory has not been upheld. Most would agree that diabetic patients are at increased risk for typical atherosclerosis. They do seem to develop a pattern of disease commonly involving the tibial arteries. Other problems in diabetic patients

also complicate the problem of foot ulceration such as some thinning of the skin of the plantar surface of the foot and an inability to deal well with infection.

## Wound Healing

Wound healing is the process by which tissues respond to an injury. The biologic process is complex and involves chemotaxis, cell replication, production of proteins, neovascularization, maturation, and wound remodeling. Wound repair and regeneration occur in an orderly and predictable manner and are under the control of growth factors, which though present in the body in only small amounts, exert a strong influence on wound repair.[13] Growth factors are polypeptides that regulate the growth, differentiation, and metabolism of cells and direct the process of tissue repair.

Wound healing occurs in three phases: inflammation, fibroplasia, and maturation. Each of these phases is controlled by growth factors. The inflammatory response begins immediately after injury. Vasoconstriction limits hemorrhage within the site of wounding.[28] Damage to the endothelial surface of arteries and veins allows blood to leak from the vessel wall, exposing platelets to collagen within the media of the vessel wall. The coagulation cascade is initiated by these platelets. Serotonin and thromboxane are released to enhance vasoconstriction locally to keep healing factors within the wound space. At nearly the same time, vasodilatation occurs at adjacent sites to allow new factors to be brought into the wound. This vasodilatation is mediated by histamine, and released by platelets, mast cells, and basophils. Vascular permeability increases to allow these blood-borne factors to enter the site of wounding.

Platelets activate the coagulation cascade through both the intrinsic and extrinsic response. The intrinsic response is mediated through Hageman factor, factor XII, as it comes into contact with collagen. In the presence of kininogen, a precursor of bradykinin and prekallikrein, factor XII activates factor XI then factor IX, then factor VIII. The extrinsic system is activated through thromboplastin formed when phospholipids and glycoproteins are released by blood coming into contact with the injured tissues. In the presence of calcium, factor VII is activated. Both the intrinsic and extrinsic systems stimulate the final common pathway leading to fibrin production and polymerization. Simultaneously, the fibrinolytic system is activated to monitor clotting so as to prevent coagulation from extending beyond the wound space.[27] It is controlled by the same factors that initiate coagulation and thus serves as a regulator of the process. Arachidonic acid is produced and serves as an intermediate for the production of prostaglandins and leukotrienes.

These intense vasodilators act with histamine, bradykinin, and complement to increase vascular permeability. Thromboxane also increases platelet aggregation and local vasoconstriction. The complement cascade is activated at the same time by platelets and neutral proteases to produce very potent anaphylotoxins, which cause mast cells to degranulate and release histamine. This process leads to margination and then migration of white blood cells into the wound space. The neutrophils are phagocytes for bacteria. Although wounds can heal without white blood cells, the risk of infection is increased. Other substances released by the inflammatory process are also chemoattractants for neutrophils, which produce free oxygen radicals and lysosomal enzymes for host defense. The neutrophils are later removed from the wound by tissue macrophages.

Monocytes migrate into the wound space by the third day and become tissue macrophages. Wounds cannot heal without the macrophage. These cells control and regulate the wound environment through the production and regulation of growth factors. These growth factors control the cellular composition of the wound as well as matrix formation and remodeling. Extracellular matrix is a variety of proteins in a polysaccharide gel composed of glycosaminoglycans and proteoglycans produced by fibroblasts. Matrix proteins may be structural such as collagen and elastin, while others such as fibronectin and laminin regulate cell adhesion. Thrombospondin, von Willebrand factor, and laminin are also adhesion molecules. Fibronectin is also a chemoattractant for circulating monocytes and stimulates their differentiation into tissue macrophages.[11]

Macrophages and fibroblasts enter the wound to begin the process of fibroplasia, the second phase of wound healing. This process begins around the fifth day following injury and may continue for 2 weeks. The inflammatory response lessens as the mediators of

inflammation are no longer produced, and those present are inactivated or removed by diffusion or by macrophages. Fibroplasia is the process of matrix formation including collagen synthesis. Angiogenesis is critical to this phase of wound healing to bring a blood supply into the wound. Fibroblasts are attracted to the wound and replicate in response to fibronectin, platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), transforming growth factor (TGF), and C5a, a product of the complement system. These fibroblasts produce proteoglycans and structural proteins necessary for wound healing. This matrix is composed of hyaluronate and fibronectin, which allow for cellular migration stimulated by chemotactic factors formed in the wound. Fibronectin is also important in epithelialization and angiogenesis.

Collagen, the major structural protein in the wound, is the most common protein in the mammalian world. Produced by the fibroblast, it is a family of at least 12 proteins, rich in glycine and proline and bound in a tight triple helix. Cross-linking between the three strands results in a very stabile molecule resistant to breakdown. The production and release of collagen from fibroblasts is controlled by growth factors such as PDGF, epidermal growth factor (EGF), FGF, and TGF-$\beta$ produced by the tissue macrophage. Once deposited in the wound, collagen is then remodeled for several years. Elastin is another structural protein in the wound present in much smaller amounts than collagen. It contains proline and lysine. It is configured as random coils, allowing both stretch and recoil. Angiogenesis is the process of capillary formation from the budding of existing capillaries after stimulation by FGF.[9, 22] These capillaries are composed of endothelial cells that migrate through the healing wound. Connections are developed between the capillaries to form a network in the wound space. This capillary network provides a pathway for new healing factors to enter the wound. The process ceases when the wound has an adequate blood supply. It appears as if the process of angiogenesis is controlled by oxygen tension and is stimulated by hypoxia. Epithelialization occurs only after granulation tissue is established. Cells migrate from the edge of the wound over the collagen-fibronectin surface. This process results in mature skin covering the wound.

Scar contracture occurs as the wound matures. During the maturation process, the scar becomes less hyperemic as blood supply is reduced. Although remodeling and wound strengthening increase for up to 2 years following injury, the total collagen content of the wound does not change. Hyaluronidase, collagenase, and elastase are key elements in wound remodeling. Hyaluronate is replaced by dermatan sulfate and chondroitin sulfate, which reduce cell migration and allow those cells already in the wound space to differentiate. Plasmin formed from plasminogen degrades fibrin. Collagenase is secreted by macrophages, fibroblasts, epithelial cells, and white blood cells and is able to break the collagen triple helix to allow remodeling. Urokinase, produced by leukocytes, fibroblasts, endothelial cells, and keratinocytes, activates collagenase and elastase.

## Growth Factors

The process of wound healing is controlled by growth factors, polypeptides that initiate cell growth and proliferation and protein production by binding to specific high-affinity receptors on the cell surface. They have the ability to stimulate mitosis of quiescent cells. They commonly have endocrine, paracrine, and autocrine function. Some are transported in plasma bound to large carrier proteins, while others affect nonadjacent cells or may have a self-regulating effect. They are produced by platelets, macrophages, epithelial cells, fibroblasts, and endothelial cells. Although growth factors are present in only minute amounts, they modulate the process of wound repair. The growth factors most commonly involved in wound healing include PDGF, TGF, EGF, FGF, and insulin-like growth factor (IGF).

### Platelet-Derived Growth Factor

Platelets that initiate the coagulation cascade in the wound are the initial source of growth factors including PDGF, TGF-$\beta$, EGF, and IGF-1. Other cells drawn into the wound space such as inflammatory cells, fibroblasts, and epithelial cells are also involved in growth factor production. Growth factors are chemoattractants for neutrophils, macrophages, fibroblasts, and endothelial cells. Macrophages release factors such as

tumor necrosis factor (TNF). Wound remodeling occurs under the control of collagenase, which is produced in response to EGF, TNF, interleukin-1 (IL-1), and PDGF. Thus, the complete process of wound repair is controlled directly or indirectly by growth factors.

PDGF is the most widely studied growth factor clinically. It is produced by platelets, macrophages, smooth muscle cells, vascular endothelium, and fibroblasts.[18] PDGF has a molecular weight of 24,000 daltons and is composed of two chains, A and B, held together by disulfide bonds in three dimeric forms, AA, AB, and BB. There is a 60% amino acid homology between the two chains. Human platelets contain all three forms of PDGF in a ratio of about 12% AA, 65% AB, and 23% BB.[3] The B chain is quite similar to the transforming gene of the simian sarcoma virus. The human proto-oncogene C-sis is similar to the viral oncogene V-cis and encodes for the B chain of PDGF. There are two PDGF receptors, an $\alpha$- and a $\beta$-receptor.[33] The $\alpha$-receptor recognizes both the A and B chains of PDGF and thus can bind to the AA form, the AB form, and the BB form. The $\beta$-receptor recognizes only the B chain, and thus binds only to the BB form and weakly to the AB form. Most cells have many times more $\beta$-receptors than $\alpha$-receptors. Cells with PDGF receptors include fibroblasts, vascular smooth muscle cells, and some microvascular endothelial cells.

PDGF is a mitogen for cells of mesenchymal origin. PDGF is a potent chemoattractant and mitogen for fibroblasts, smooth muscle cells, and inflammatory cells. It also acts with TGF-$\beta$ and EGF to stimulate mesenchymal cells. Although PDGF is produced by endothelial cells, they do not respond to PDGF but work in a paracrine manner to stimulate adjacent vascular smooth muscle. Smooth muscle cells also act in an autocrine fashion and produce PDGF.

PDGF is stabile to extremes of heat, a wide range of pH, and degradation by proteases. Platelets are the largest source of PDGF in the human body. There are no cases of a human PDGF deficiency state, perhaps suggesting that PDGF is critical to the survival of the individual.

PDGF has been isolated from the $\alpha$-granules of platelets and has been produced through recombinant DNA technology. In animal models, it has been shown to improve the breaking strength of incisional wounds in rats when applied topically even as a single dose.[23] PDGF accelerates wound healing; however, by 3 months, there is no difference in wound healing as compared with untreated wounds, suggesting that wound healing stimulated by PDGF is quite similar to normal healing. Wounds treated with PDGF had a marked increase in neutrophils, monocytes, and fibroblasts in an animal model.[21] This cellular response leads to an increase in granulation tissue production. Similar findings were observed in a rabbit ear excisional wound model. Despite the fact that PDGF does not have a direct effect on keratinocytes, wounds in animals were shown to have an increase in epithelialization. This is due to the influence of macrophages and fibroblasts attracted into the wound by PDGF. Wounds in animals treated topically with PDGF have an increase in neovascularization, although PDGF does not directly stimulate endothelial cells. Again, this is likely related to the influence of other cells attracted into the wound by PDGF. Thus it appears that PDGF accelerates wound healing by accelerating the normal responses. The healed wounds appear to be normal in all aspects.

PDGF has been studied in several clinical trials. The effectiveness of recombinant human PDGF-BB in healing decubitus ulcers was evaluated in patients treated and followed for 28 days.[24] There appeared to be greater reduction in wound closure in patients treated with PDGF. In another trial, patients with decubitus ulcers were treated with PDGF or placebo for 1 month.[20] The ulcer volume was significantly reduced in the PDGF-treated patients. No significant toxicity related to PDGF was noted in either study. A randomized, prospective, double-blind trial of recombinant human PDGF-BB was performed in patients with diabetic neurotrophic foot ulcers.[29] Patients were treated with PDGF (at a dose of 2.2 $\mu g/cm^2$ of ulcer area) in vehicle, carboxymethylcellulose, or vehicle alone for up to 20 weeks or until complete wound closure had been achieved. All wounds had been present for at least 8 weeks. All patients were free of infection and had an adequate blood supply as demonstrated by a transcutaneous oxygen tension ($TcPo_2$) of a least 30 mm Hg. Wounds were debrided prior to entry into the study and as needed during the trial. Forty-eight percent healed using PDGF, while only 25% healed using vehicle alone ($p < 0.01$). The median reduc-

tion in wound area was 98.8% for PDGF-treated patients but only 82.1% for those treated with vehicle. There were no significant differences in incidence or severity of adverse events in either group. This was the first study to suggest that a growth factor, and specifically PDGF, could be applied topically and be effective and safe in promoting the healing of chronic wounds in humans. In another trial using recombinant human PDGF in the treatment of patients with diabetic foot ulcers, PDGF-BB was found to increase the incidence of complete wound closure by 43% as compared with placebo ($p = 0.007$).[34] It also decreased the time to achieve complete wound closure by 32% ($p = 0.013$) when compared with the placebo gel. Over 1,000 patients treated with PDGF have been reported. It appears to be safe and efficacious and is now approved for use in the treatment of diabetic foot ulcers. It has been noted that those patients receiving the best wound care healed better when treated with PDGF. Debridement was found to be critically important.[30] The benefits from PDGF will not be achieved if the wounds are not treated properly.

## Vascular Endothelial Growth Factor

Vascular endothelial growth factor (VEGF) is quite similar to PDGF. It has a molecular weight of 45,000 daltons. Although it has a 24% amino acid homology to the B chain of PDGF, it binds different receptors than PDGF and has different actions.[17] VEGF is a potent mitogen for endothelial cells but not for fibroblasts or vascular smooth muscle cells as is PDGF. Thus, VEGF is angiogenic and plays a role in wound healing through this mechanism. There has been interest in using VEGF to stimulate the development of collateral vessels in patients with symptomatic arterial ischemic disease. VEGF may be delivered to the ischemic area as a protein or through gene transfer.

## Fibroblast Growth Factor

Fibroblast growth factor is a series of heparin-bound growth factors.[10] There are two forms of this growth factor, acidic FGF (aFGF) and basic FGF (bFGF). Both are single-strand molecules with molecular weights of 15,000. There is a 50% amino acid homology between the two molecules. The binding of these molecules to heparin or heparan sulfate may protect them from enzymatic degradation. Both aFGF and bFGF are found in the extracellular matrix in the bound form. Matrix degradation proteins may act to release aFGF or bFGF. Acidic FGF is similar to endothelial cell growth factor, while bFGF is similar to endothelial cell growth factor II. Both aFGF and bFGF are similar to keratinocyte growth factor. FGFs are produced by fibroblasts, endothelial cells, smooth muscle cells, and chondrocytes and are mitogens for cells of mesodermal and neuroectodermal origin. FGFs are also potent mitogens for endothelial cells and act as angiogenesis factors by stimulating growth of new blood vessels through proliferation of capillary endothelial cells.[8] In addition to endothelial cells, FGFs can stimulate fibroblasts, keratinocytes, chondrocytes, and myoblasts. Multiple different FGF receptors have been identified. They appear to have a similar function. To date, there are no clinical trials that have proven FGF to be of benefit in clinical wound healing.

## Keratinocyte Growth Factor

Keratinocyte growth factor (KGF) is related to the FGFs. It is a protein with a molecular weight of 28,000 and has a significant amino acid homology with the FGFs.[6] Although KGF is found only in fibroblasts, it stimulates keratinocytes, not fibroblasts. It may share a receptor with FGF. At this time, there are no clinical trials reported using KGF, and its role in human wound healing remains to be defined.

## Transforming Growth Factors

TGFs are composed of two polypeptide chains, $\alpha$- and $\beta$.[26] TGF-$\alpha$ has a 30% amino acid homology with EGF. It received its name because of its ability to reversibly stimulate the growth of cells. Cancer cells do this as well. TGF-$\alpha$ is produced by macrophages, keratinocytes, hepatocytes, eosinophiles, and other cells. TGF-$\alpha$ and EGF are mitogens for keratinocytes and fibroblasts but TGF-$\alpha$ is a more potent angiogenesis factor. Both TGF-$\alpha$ and EGF bind to the EGF receptor but their specific actions may be different partly due to differences in their binding to

the receptor. To date there have been no clinical trials of wound healing with TGF-$\alpha$.

TGF-$\beta$ has no amino acid homology with TGF-$\alpha$ or any other growth factor. TGF-$\beta$ is a molecule with many different functions and can stimulate or inhibit the growth or differentiation of many different cells. It is, thus, in some respects a master growth factor. There have been three different forms of TGF-$\beta$ isolated, TGF-$\beta$1, TGF-$\beta$2, and TGF-$\beta$3.[19] Just as there are three forms of TGF-$\beta$, there are three receptors, although the three are not equally important. The actions of the three different forms of TGF-$\beta$ are very similar although not exactly the same. TGF-$\beta$s have a molecular weight of approximately 25,000. TGF-$\beta$ is a group of proteins that can reversibly inhibit growth of cells, especially those of ectodermal origin. TGF-$\beta$ is produced by a variety of cells including platelets, macrophages, fibroblasts, keratinocytes, and lymphocytes. Nearly all cells have receptors for TGF-$\beta$ and have the potential to respond to it. It appears as if TGF-$\beta$ is the most widely acting of the growth factors. The TGF-$\beta$s are potent stimulators of chemotaxis in inflammatory cells and stimulate cells to produce extracellular matrix. These characteristics make the family of TGF-$\beta$s important to the wound healing process. There have been trials of TGF-$\beta$ in the treatment of psoriasis, but as yet, there are no trials reported using TGF-$\beta$ in human wound healing.

## Epidermal Growth Factor

EGF is a small molecule quite similar to TGF-$\alpha$, with a molecular weight of 6,200 daltons. EGF is produced by the platelet and is present in large amounts in the early stages of wound healing. EGF is produced by the kidney, salivary glands, and lacrimal glands; therefore, it is found in high concentrations in urine, saliva, and tears.[7] EGF promotes epidermal regeneration and corneal epithelialization, and increased the tensile strength of wounds in animals. EGF increases wound healing by stimulating the production of proteins such as fibronectin and the migration of epithelial cells. Although EGF does not stimulate collagen production, it increases the number of fibroblasts in the wound. These cells produce collagen and improve the wound strength. EGF has a common receptor with TGF-$\alpha$. EGF has been studied in a randomized trial of skin graft donor site healing. Treatment of donor sites with silver sulfadiazine containing EGF accelerated epidermal regeneration compared with sites treated with silver sulfadiazine alone.[5] EGF reduced the healing time by 1.5 days. Although these results may not be clinically significant, this was the first trial to suggest a benefit from treatment with an isolated growth factor in human wounds. EGF was also used in a prospective open label trial in patients with chronic wounds.[4] Chronic wounds were treated with silver sulfadiazine. In this crossover dosing, those who did not heal were then treated with silver sulfadiazine containing EGF. Many of the patients improved. The results from these studies suggest that EGF may be beneficial in wound healing, although there is not adequate proof as yet to confirm this premise.

## Insulin-Like Growth Factors

IGFs, also called somatomedins, are proteins sharing a 50% amino acid homology with proinsulin. They have insulin-like activity. The two forms of this growth factor, IGF-1 and IGF-2, are both secreted as large precursor molecules, which are cleaved to an active form.[25] IGF-1 is also called somatomedin-C while IGF-2 is simply somatomedin. Many tissues synthesize these growth factors. IGF-1 can be found in the liver, heart, lung, pancreas, brain, and muscle.[2] Although produced by a number of tissues, IGF-2 is particularly prominent during fetal development and plays a significant role in fetal growth. IGF-1 and IGF-2 have separate receptors. The actions of pituitary growth hormone are mediated through IGF-1. Pituitary growth hormone stimulates cell differentiation and the production of IGF-1. IGF-1 then causes cell division. IGF-1 is produced mainly in the liver. It is found in high concentrations in platelets and is released into the wound space when clotting occurs. Levels of IGF-1 and IGF-2 depend on multiple factors, such as age, gender, nutritional status, and hormone level. Growth hormone regulates IGF-1 and IGF-2 levels as does prolactin, thyroid hormone, and the sex hormones. Elevated levels of somatomedins have been identified in patients with acromegaly.

IGF-1 and IGF-2 are anabolic hormones that stimulate the synthesis of glycogen, pro-

tein, and glycosaminoglycans. They increase the transport of glucose and amino acids across cell membranes. They also increase collagen synthesis by fibroblasts. There is no clinical evidence to suggest these growth factors are of benefit in treating wounds.

As previously described, the process of wound healing is complex and involves platelets and macrophages. In the first 2 days following injury, platelets control the wound space by way of growth factors that they produce and release. Following this period, this function is taken over by macrophages. Within the $\alpha$-granules of the human platelet are multiple growth factors that are released when platelets are activated and degranulate. These include PDGF, TGF-$\beta$, FGF, EGF, platelet factor 4, platelet-derived angiogenesis factor, and $\beta$-thromboglobulin.

## Platelet Releasates

A purified platelet releasate has been prepared by stimulating human platelets to release the contents of their $\alpha$-granules by using thrombin. Use of a platelet releasate in wound healing has several advantages. First, the growth factors that are released are identical to and in the same proportion as the growth factors normally brought in the wound space by the platelet. Second, preparation of a platelet releasate is relatively easy and inexpensive, as the platelets can be harvested from peripheral blood. They readily release the contents of their $\alpha$-granules when stimulated with thrombin. As growth factors are preserved in banked blood, large amounts can be retrieved from the platelets of pooled human blood. There are, however, disadvantages to using a platelet releasate. Not all growth factors promote healing of a wound. It seems reasonable to assume that there is a signal for wound healing to stop. It is likely that this, too, is growth factor related. Since the proper concentration of a platelet releasate is uncertain, a platelet releasate might concentrate factors promoting healing as well as those that trigger the wound healing process to end. Another major disadvantage of such a preparation is that there is the possibility of transmission of an infectious agent from the platelet donor. This could be minimized if the releasate were harvested from a single donor.

## Clinical Trials

There have been several reports using a platelet releasate in wound healing. A preliminary report described the use of an autologous platelet releasate in six patients with chronic lower extremity ulcers from connective tissue diseases with minimal benefit. A homologous platelet releasate was used to treat 11 patients with leg ulcers from diabetes and eight patients with leg ulcers secondary to chronic venous insufficiency.[31] No benefit was observed from the treatment with a platelet releasate. In managing these patients, treatment of the underlying disease was not addressed. Growth factors cannot be expected to improve wound healing unless they are applied in a comprehensive wound care program that addresses the underlying etiology of these wounds such as diabetes, venous hypertension, or ischemia. In another trial, 49 patients with chronic nonhealing cutaneous wounds were treated with an autologous platelet releasate.[15] There was some improvement in achieving complete wound healing. This was the first clinical trial to suggest a benefit from a platelet releasate applied topically to promote the healing of chronic wounds. A randomized trial comparing platelet releasate versus a placebo was conducted in patients with ulcers secondary to diabetes, peripheral vascular disease, venous insufficiency or vasculitis.[14] Although this study suggested a benefit from the treatment, the growth factor preparation was added to microcrystalline collagen, a potent stimulator of platelets. The exact contribution from the collagen to the healing of these wounds was not clear. Two other trials suggested a benefit from a platelet releasate. In one trial, patients were treated for 3 months with saline and silver sulfadiazine. Only 3 of the 23 lower extremity wounds healed; however, when the platelet releasate was then applied, the remaining ulcers healed.[1] Thirteen patients with diabetic neurotrophic ulcers were enrolled in a randomized trial of a platelet releasate versus saline placebo.[32] A benefit was seen in those treated with the platelet releasate. By 20 weeks of therapy, five of seven patients healed using the platelet releasate, whereas only two of six patients healed using the saline placebo. By 24 weeks of treatment, another three of six patients in

the control group healed, suggesting that the platelet releasate stimulated more rapid healing but did not result in a greater proportion of healed wounds. Another study of 70 patients suggested a similar benefit from a platelet releasate.[12]

Despite this evidence that platelet releasates are of benefit, another trial observed very different results using a homologous platelet releasate. In a randomized, prospective, double-blind, placebo-controlled trial, topical platelet releasate was applied to leg ulcers due to diabetes, peripheral vascular disease, or chronic venous insufficiency. Wounds treated with the platelet releasate worsened, while wounds in the control group improved.[16] This study not only suggested no benefit from a platelet releasate over standard care in the management of lower extremity ulcers, but in fact intimated that a platelet releasate might be detrimental to wound healing.

Although the results of the clinical trials using platelet releasates have been varied, there is some evidence to suggest that they may be of benefit applied topically to lower extremity wounds. However, the inconsistency of the results as well as the concern about transmission of infectious agents in using a homologous preparation leaves their role in human wound healing undefined. Molecules other than growth factors may play a role in the healing of wounds, especially in patients with diabetes. Mice deficient in inducible nitric oxide synthase (iNOS) had a slower rate of healing of full-thickness wounds than normal animals.[35] When given iNOS, the rate of healing returned to normal.

## Conclusion

In conclusion, growth factors control the growth, differentiation, and metabolism of cells. Thus they control wound repair and maturation, although there are only a limited number of reports where growth factors have improved wound healing. One isolated growth factor, PDGF, does improve healing in diabetic ulcers and is approved for that indication. It is likely that the actions and benefits of growth factors will be defined further. This will allow health care providers to control the wound environment to achieve complete and durable wound healing in patients.

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Attorney's Docket No. 035626/234825 PATENT

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

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|---|---|---|---|
| In re | Anton-Lewis Usala | Confirmation No.: | 7087 |
| Appl. No.: | 09/870,414 | Art Unit: | 1614 |
| Filed: | May 30, 2001 | Examiner: | To Be Assigned |
| For: | METHOD OF TREATING CHRONIC ULCERS | | |

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

**SUPPLEMENTAL INFORMATION DISCLOSURE STATEMENT**
**CITATION UNDER 37 C.F.R. § 1.97**

Sir:

Attached is a list of documents on form PTO-1449 together with a copy of each identified document. It is requested that the Examiner consider these documents and officially make them of record in accordance with the provisions of 37 C.F.R. § 1.97 and Section 609 of the MPEP. By submitting the listed documents, Applicant in no way makes any admission as to the prior art status of the listed documents, but is instead submitting the listed documents for the sake of full disclosure.

It is not believed that any fees are necessary to allow consideration of this paper. However, if any fees are due, Applicant hereby authorizes any fees to be charged to Deposit Account No. 16-0605.

Respectfully submitted,

Christopher M. Humphrey
Registration No. 43,683

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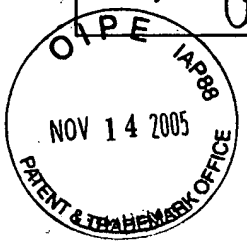


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Substitute for form 1449A/PTO
(Revised 10/2001)

# INFORMATION DISCLOSURE STATEMENT BY APPLICANT

*(Use as many sheets as necessary)*

Sheet 1 of 1

| Complete if Known | |
|---|---|
| Application Number | 09/870,414 |
| Filing Date | May 30, 2001 |
| First Named Inventor | Anton-Lewis Usala |
| Group Art Unit | 1614 |
| Examiner Name | To Be Assigned |
| Attorney Docket Number | 35626/234825 |

## U. S. PATENT DOCUMENTS

| Examiner Initials* | Cite No. | Document Number<br>Number - Kind Code (if known) | Publication Date MM-DD-YYYY | Name of Patentee of Applicant of Cited Document | Pages, Columns, Lines, Where Relevant Passages of Relevant Figures Appear |
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| | 58 | 6,046,160 | 04-04-2000 | Obi-Tabot | |
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| | 60 | 6,372,494 B1 | 04-16-2002 | Naughton *et al.* | |
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## FOREIGN PATENT DOCUMENTS

| Examiner Initials | Cite No. | Foreign Patent Document<br>Country Code - Number Kind Code (if known) | Publication Date MM-DD-YYYY | Name of Patentee or Applicant of Cited Document | Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear | T |
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*Examiner: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant. RTA 2155197v1